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EDITORIAL

THE NOISE OF MOLECULES

TRAINED electrons are the latest toys and tools of Science. Shadows, and the whispers of ghosts, impel an invisible electric giant to heave a monstrous bridge high in the air, to swing an untouched door, to count a myriad persons passing a robot's vitreous eye, and to accomplish marvels and miracles in a most effortless and routine way.

Sounds never heard before are amplified to a painful audibility. Our heretofore limited senses are spanned and spread to boundless possibilities. The heartbeats of gnats, the pulsing of sap in the April-stretched hickory, the human voice, far flung from the frozen wastes of Antarctica, come clear to our ears, on the wings of obedient electrons.

Once, we seemed grateful that the all-merciful Intelligence who handles our dust, our deeds and our destinies limited our senses to a rational range—acceptable to our limited equipment.

And we were happy with normalcy, nor even envied the cubist, his angular complex—nor the color-mad artist, his passion for splash. We accepted the so-called radicals and long-hairs as persons whose special senses were developed finely or queerly away from the normal.

A visit to an exhibition of ultra-modern art made us more than happy with normalcy. When an artist's sense of sight becomes so enamored of angles that curves hide with sudden shame—is it any wonder that his delineation of "A Street Scene in Simla"—looks to normal beings like a score of clothespegs dancing on a corrugated wash board.

Yet it is a natural broadening of this same sense of vision and a kindred acuity of form and of color interpretation that makes a Turner or a Michaelangelo.

Then consider the range of our auditory equipment. Our cranial receiving sets can not tune in beyond certain high notes and below certain low ones, exactly as with our ordinary radio sets we cannot accommodate beyond and below certain wave lengths.

Yet in this field are again the few who are abnormally talented in their range of sound appreciation, and in their ability to compound these sounds into song or prose or poetry exactly as the artist weaves his canvas out of colors, and the sculptor his statue from clod of mud or marble.

But with the sense of sound, too, while thinkful for the God-given limits of tonal appreciation—who has not envied those who like the Maid of Rouen had the capacity to hear strange voices and to be moved by them?—sweet Joan of France who—

> "Heard a voice that no one else could hear
> E'en tho the hillsides echoed, loud and clear."

And the bells of London Town that clanged only for Dick Whittington!

But when it is recalled that a guilty conscience can also spread the hearing and other sensibilities—as it did with persons of fancy, such as Lady Macbeth and the Abbot of Aberbrothoc with the bells on the Inchcape Rock, and with persons of fact such as Cain and Judas, our coefficient of thankfulness for normalcy again increases.

Meticulously indeed has the sound recording device in us been so arranged and developed that it registers only that span of tones acceptable to the organization that encompasses it. Had our ears been made sensitive to the noise of molecules—a day in a forest hearkening to atoms agog in growing trees would set us crazy for the rest of our lives.

Listen to what Huxley, in speaking of the stirring activities going on in plant cells, says: "The wonderful noon day silence of a tropical forest is, after all, due only to the dullness of our hearing; and could our ears catch the murmur of these tiny maelstroms, as they whirl in the innumerable myriads of living cells which constitute each tree, we should be stunned, as with the roar of a big city." And whoever has heard the radio receiving set, after it catches the infinitely small electric impulses and whips them up into sounds that fill a large hall can appreciate the fullness of his statement. For sounds more silent than the farewells of ghosts can be amplified so

with this new mechanism that the human ear is paralyzed with their turmoil.

But there are queer artists in tonal effects exactly as there are queer artists of form and color. For in the realm of sound interpretation too, you will find the "musical" (?) cubists and futurists—who only see the odd angles of music and fail to get the curves and concord of sweet sounds.

Nor are these two senses alone in their limitations, for happily the rest of our senses are likewise governed. There are odors and flavors—and touch vibrations too fine or too coarse for our limited senses to appreciate. Thank goodness for a dignified normalcy in these directions, too.

Hearken to what Dr. Mackenzie of the medical faculty of Edinburgh has to say about our sense of smell. He insists that the olfactory equipment of man is scarcely better than that of the amoeba. As a matter of fact it is not as highly organized as is the predatory smell sense of as low a creature as the pseudopodic vampyrell, which can apparently distinguish its special diet of algeal spirogyra by smelling it a hundred bacterial miles away.

"If in comparison with Fido, our sense of smell were as highly developed as our vision, we would in truth be sitting on top of the world. We could smell around corners. We should not have to resort to guessing at the strength of an opponent's hand."

Nor is it nearly as ridiculous as it sounds. Look what the butterflies can do! You can insulate certain of them—polyphemus moth is one, and the commonest one hereabouts—with steel, glass or what you will, and its mate can smell it out,—a fact which has led scientists to the interesting conclusion that whereas most odors travel by emanation, by waves of the odorous principle itself, others are transmitted in the same way that sound waves travel. Man is, presumably, insensible to these latter, just as he is to the direct contact of the sounds which are caught up in his radio and reduced to intelligible terms.

But the sense of smell is in a category quite distinct from those of sight and sound, for the images it presents to the human brain are, in spite of their dual nature quite fugitive. The most that a whiff of a smell can do with us is to remind us of the last time we smelled it.

So we may register our thanks for a fundamental limitation to our sense of smell and for the fact that there is no special incentive to develop its acuity. In other words the nose knows enough.

But all of this only leads us to a poignant, pertinent question—In this hectic development of the new forces now intrigued by man—is there a danger that we may all of us—someday—"lose our senses"?

IVOR GRIFFITH.

Grapefruit Oil. Drug and Cosmetic Industry. 36, 215, (1935). Florida grapefruit oil, which is new to commerce, is finding favor for flavoring purposes and has possibilities in perfumery. The oil is obtained from the Florida common or Duncan type of grapefruit by the Pipkin process, in which the peel passes between knurled rollers. The oil is separated from the emulsion by a high speed centrifuge. Analysis gave the following average constants: Specific gravity, 0.8563; optical rotation $[\alpha]_D^{20°}$ 93.28 degrees; refractive index, 1.4758; aldehydes as octyl and decyl aldehydes, 1.67 per cent. The composition corresponds to approximately 90 per cent. of limonene, 2 to 3 per cent. of oxygenated volatile constituents and sesquiterpenes and 7 to 8 per cent. of waxy materials. Octyl and decyl aldehydes are present, as also is citral and methyl anthranilate in small quantities. Geraniol and octyl alcohol are present both as free alcohols and as acetates. Cadinene was also identified. A terpeneless oil was also made in which the "turpentine" taste was eliminated.

Test for Peroxide in Ether. A. Castiglioni. *Ann. Chim. appl. Roma., 24,* 209 (1934). Through *Quart. Journ. Pharm. Pharmacol. 7,* 692 (1934). Evaporate 1 or 2 cc. of ether in a porcelain dish, add 1 drop of alcohol (95 per cent.), 1 drop of benzaldehyde and 1 drop of concentrated sulphuric acid. If peroxide is present a bright eosin-red color is produced. This is the cause of the color produced in Melzer's test for pictrotoxin, which is not due to the latter substance.

ORIGINAL ARTICLE

DAPHNIA PROPAGATION FOR EXPERIMENTAL USE*

By Dr. Arno Viehoever

Occurrence in Nature

THE small crustacean daphnia furnishes one of the important foods for our fishes in the open inland waters and in fisheries, as well as for tropical fishes in our homes. Some species or another of daphnia appears to occur in almost any locality where waters collect in pools exposed to the sun and rich in food substances, providing in turn for active micro-life. The most likely breeding places or localities favoring their accumulation are the somewhat stagnant drainage pools on farm lands, where farm animals, cows and horses, ducks, geese, chickens or pigeons—through their excreta—enrich the water for the growth of water-plants, minute and large, and water-animals, infusoria and water insects. Ditches, ponds, inlets, bays or small lakes, into which drain the dung liquors of the farm or the sewage of the city population or in which we find animal corpses, including fish in the process of decay, are promising spots where large numbers of daphnia may be collected.

Factors in Laboratory Propagation

For the propagation of daphnia magna in the laboratory for experimental use (1) the conditions found favorable in nature may be simulated. There are certain factors which play an important role, namely: 1—the purity and quantity of water supplied in the culture medium; 2—the extent of oxygen available to the animals; 3—the degree of temperature of the culture water; 4—the intensity of the light to which the cultures are exposed; 5—the kind of containers in which they are kept; 6—the reaction of the culture medium; 7—most essential—the amount and nature of food—, the environment created by the application of judicious care; 8—the exclusion of enemies—and, finally, the selection of a pure line stock of giant daphnia.

*From Philadelphia College of Pharmacy and Science, as a contribution from the Laboratory for Biological and Biochemical Research, supported by Mr. Wm. H. Gross.

1. Water

The selection of suitable water supplies is of prime importance for the success of the culture medium. We have found ordinary water taken from a well-balanced aquarium usually satisfactory. Of course this is an emergency measure from the point of view of preparing an ideal standard culture medium. We have also used with success water from a nearby large open pool, (in which many water-plants grew), after filtration through pure filter paper or a Berkefeld filter. Tap water, particularly when recently chlorinated, is generally either artificially aerated or placed aside for several days in open containers to furnish a satisfactory water supply for breeding. The suitability of the water to be used, whether tap, well or spring water, or water from a pond or lake must be tested. It may be expedient to collect a larger amount from a satisfactory source as an inland sea and, after careful filtration, keep it in suitable containers.

Distilled Water. The use of commercially prepared distilled water has, as a rule, been unsatisfactory, due to the presence of traces of heavy metals from the distillation apparatus used. It appears best, particularly for special controls, to use water—distilled, preferably twice, in special glass or quartz apparatus,—or condensed in tin coolers. It is obvious that, for the ideal standard medium, only pure distilled water and pure chemicals should be used.

Amount. We have repeatedly kept daphnia for several days—and even observed the birth of young—in as little as 10 drops or ½ cc. of culture water. In fact, on moist gauze, providing merely a film of moisture to the animals, these can be kept alive for a day or more, depending upon the temperature and satisfactory condition of the water film. The confined conditions become ultimately unsatisfactory, leading to the suppression of the maturing of eggs (2)—and eventually premature death. McArthur and Baillie,(3) having experimented under somewhat comparable conditions, but varying the amount of culture liquid from 0.6 cc. to 150 cc., conclude that even 40 cc. allowance of culture fluid is below the optimum for daphnia magna. For optimum results as much as 100 to 150 cc. per individual is recommended.

Dehydration. Upon gradual removal of the water the muscles showed the effect first. The heart kept on pumping even after the tissues had shrunk—due to serious loss of body fluids. Recovery

was possible in a number of individuals even after the heart had stopped for fractions of a minute. (4)

Color and Clarity. The more or less brownish-yellow color has been used by fish breeders as Hayford (5) of the New Jersey State Fisheries—as an indicator of major importance for the progress of the desired pollution. Also the statement, "The more foul the ponds become, the better the production of daphnia", is one of his conclusions. Of course, to avoid continued pollution and the accumulation of toxic waste in the water, he draws off the ponds and resets them at least once a month. As a rule we have obtained best results with manure culture water which was colored yellowish or brownish yellow from organic food substances dissolved in it. Marked turbidity is usually a sign of excessive pollution,—at least for laboratory cultures without circulation of culture water. After many initial failures in attempts of continuously breeding daphnia in water, markedly turbid or distinctly cloudy with the growth of micro-organisms, we now only breed in fairly clear culture water, particularly when the populations are not dense, and the cultures are not exposed to direct sunlight. Some culture media, containing blood in high dilution, or soybean flour, dried- and malted milk (Horlick's), oatmeal, or special cultures of bacteria and yeast, remained practically colorless.

2. Oxygen

It is generally recognized that a daphnia culture, particularly in dense growth, consumes oxygen heavily. The collectors, knowing that warm water holds less oxygen than cold water, place cakes of ice in the pails, teaming with daphnia, during warm weather, to insure the live delivery of the maximum number in the catch. On account of the large and highly efficient breathing system furnishing oxygen even in highly polluted water, or at the bottom of pools to the daphnia, they may exist on very little oxygen indeed. In some of my experiments some daphnia lived for days in closed ampules.

If the feeding is not effected with autotrophic protozoa or algæ,—or is not carried on in the presence of higher water plants, furnishing oxygen in the assimilation process, provision for access of air or oxygen must be made. Shallow cultures, to upward of 6 inches, as a rule have succeeded better in our laboratory, than cultures with a depth of 10 inches or more in long, narrow containers. We have used with beneficial results the aspiration furnished by the small, inexpensive magnetic airpumps, designed for use in aquaria.

3. Temperature

Daphnia Magna, a native of Central Europe and also found in Canada, where it must be able to survive cold weather, is now bred in open pools—in certain State Fish Hatcheries as that of New Jersey. Hayford found it advantageous to place his daphnia during the winter in a heated building and even to heat, in cooler room temperatures, the culture aquaria by electric heating units. In his experience the water with daphnia should not get too cold; daphnia should not be poured from warm water into cold, to avoid sudden temperature changes, fatal to daphnia. Thus, too, the temperatures should be uniform, if it becomes necessary to start new cultures.

Daphnia, nevertheless, readily withstands temperatures between a range of approximately 5° and 30° C.(3) In my own experiments it was possible to place animals in freezing temperature as low as approximately —6° C. Of 16 animals, thus frozen, at least 4 recovered.(4)

The production of male forms, as a result of lowering the temperature in the culture water, has been reported in earlier work. Placing daphnia repeatedly and for varied times in the refrigerator has, however, not produced males. The fact that males are obtained in increased numbers in the fall is obviously a result of food shortage, rather than of lowering of the temperature.(6) (7)

As a rule we cultivate our stock at the temperatures of ±21° C.—room temperature—and aim to retain that temperature for breeding as well as for experiments throughout the year.

Yerkes, studying the effect of heat upon the movements of daphnia (pulex) concluded that it is negatively thermostatic at a temperature of 28° C., having moved from water of that temperature in a trough to the water cooled down to 25° C. The irregular wandering, rather than the direct movement, he attributes to differing intensities of stimulations effecting different regions of the animal body. The heat accompanying the light from a 16 candle-power filament lamp had no noticeable influence upon the direction or rate of the movement of daphnia.

4. Light

The cultures of daphnia are best kept in diffuse North light, particularly when fed on autotrophic organisms and in dense populations requiring oxygen. Direct sunlight keeps the growth of bacteria and

protozoa somewhat in check, thus preventing excessive growth in certain culture media, although stimulating the growth of algae.

Daphnia species, as may be readily observed, are attracted by light. Yerkes experimenting with Daphnia pulex, found that it responds equally strong to intensities of light from about 0 to 100 candle-power. Exposure to a light of 16 candle-power for one hour caused a gradual increase in rate of movement and in the sensitiveness of light. The daphnia, which had moved as much as 2000 cm. in one hour, showed no evidence of fatigue.

5. Culture Flasks

In most cases culture flasks of ordinary glass, even empty ink bottles, jelly and mayonnaise jars have proven satisfactory. Only in special cases, particularly for check purposes, containers of pyrex, Jena or quartz glass were found necessary. In emergency a protection against solution of alkali from the inner wall of ordinary glass containers may be secured by covering the inside wall with molten paraffin, heated well above its melting point and finally adequately cooled to obtain uniform distribution. The culture flasks and all other containers and tools or instruments should be carefully cleaned and kept separate from fixing materials and other poisons. Containers for microcultures as well as for mass cultures should be protected from excessive evaporation and infection.

For mass cultures we have used with particular satisfaction, wide-mouth gallon jars used for the commercial packing of mayonnaise, and thus available at low price,—covered with perforated, lacquered, filterpaper lined, tops of tin cans. Hayford, in the New Jersey State Fisheries, uses 7 quart glass perch hatching jars for winter breeding. For cultures, greatly limited in numbers of daphnia, half-filled wide-mouth jars are preferred, holding—according to need—¼, ½ or 1 pint—or 1 quart, also, covered with perforated tops as indicated.

6. Reaction of Culture Medium

The reaction of the culture water, measured by the concentration of hydrogenions, has a great influence, now well recognized, upon the nature of the micro-organisms growing in them. While many molds and yeasts are usually found in acid media, bacteria, as a rule, thrive better in alkaline media. It is an interesting observation that most natural ponds react alkaline [ph. 7.6].(3) and that daphnia

evidently prefer a definitely alkaline culture medium. While daphnia will grow at an alkalinity of as high as ph. 8.1-8.5, we provide for an even alkalinity of ph. 7.8-8 by simply adding an excess of calcium carbonate, either in powder or in the form of marblestone, crushed or in pieces. We are also experimenting with small amounts of urea, changed by urea bacteria into ammonium carbonate.

7. Food

The most important factor in the laboratory breeding of daphnia is the selection of food and the procedure of feeding. It is generally recognized that food must be always available, but that an over-abundance is detrimental, if not fatal. Lack of food through infrequent feeding—in contrast—may lead to the development of winter-eggs—"Ephippa", suitable for storing and shipping and subsequent germination only. To effect an artificial cultivation one must succeed: first, to keep the animal alive; second, to nourish it sufficiently; third, to breed it, continually through the various stages of life in successive generations; fourth, to cultivate it in an environment, removed from the natural habitat.

The ideal aim of cultivation in an artificial medium is to satisfy completely the needs of the animal for life, growth and progenation with a food that is adequate, always available, stable and of uniform quality. The ideal pure culture implies breeding the desired number of living animals in an artificial medium supplied with an artificial food. Where the purpose requires it, where the time is available for the additional care, ideal artificial conditions should be furnished.

Types of Cultures

Four type cultures may be differentiated as:—

1. *Raw Cultures:* Mixtures of organisms, changeable and uncontrollable, serving as a source for pure cultures. Such cultures of impure and of changing composition—both chemically and biologically—are obtained upon suspension of manures, fertilizer, blood, cereals, etc., in water, or upon setting aside hay infusions or meat bouillon. In suitable concentrations they furnish culture media for a host of micro-organisms as bacteria or protozoa.
2. *Mixed Species Pure Cultures:* Such cultures may readily be obtained from the raw cultures of type 1, if among the organisms

are those that may serve as living fodder organisms for a pure strain of organisms specially introduced. Thus the desired organism, daphnia in our case, may be introduced as a pure culture, free from related forms, to a raw culture, obtained as indicated, provided that there is present in the culture, or that there can be added to it the same or similar substances, sustaining the life of the micro-organisms, serving—at least in part—as living fodder.

3. *Species Pure Cultures:* These cultures represent only the desired culture organisms and the living fodder organisms in pure culture. As we shall mention later in detail, such cultures may now be prepared for daphnia, living from food, exclusively furnished by certain specific living bacteria.

4. *Ideal Pure Cultures:* Such cultures, consisting merely of the desired culture-organism and non-living food substances, are commonly obtained in the cultivation of bacteria and fungi. Some of these forms will even live on media of definite chemical composition, permitting accurate and absolute standardization of the food. Many attempts to grow daphnia as ideal pure cultures, in the absence of living organisms, have thus far not been successful.

Types of Food

As daphnia seemingly require living food, we must prepare it from raw cultures or supply it under suitable conditions with pure cultures. Banta, (8) as one of the first in this country, ascertained that daphnia may be fed: 1, upon a mixture of various protozoa and protophytes from the sediment of ponds, containing an abundance of organic matter; 2, upon bacteria; or 3, upon certain unicellular green algæ. For years he utilized for his cultures the slightly colored water of a pond, collecting with it much of the fluffy sediment floating upon the bottom. This sediment, to prevent contamination of the laboratory stock cultures, was strained through a silk bolting cloth and distributed, mixed with the culture water, into wide-mouth culture flasks, holding about 200 cc., so that they were about one-half filled.

His experience with culture water thus collected, however, made him conclude (and other workers as well as ourselves agree) that, 1, the mixture of water and sediment found in most ponds does not constitute a proper culture medium; 2, it is difficult to find a pond

TABLE I

NATURAL MANURES

Natural manures	Amount	Additions	Amount	T. ° C.	Applications	Results	References	Year	Remarks
Horse manure, fresh	0.25%			25° C.	Weekly or bi-weekly		Naumann (9)	1925	In diffuse light
Horse manure, fresh	0.15%			25° C.	Weekly in daily proportions		Naumann	1925	In diffuse light
Horse manure, fresh	Bag-size of hen's egg	Decayed lettuce leaves	Some	25° C.	Bag hung into water in a 7-quart jar	Very satisfactory	Hayford (5)	1929	
Horse manure, liquor (1:5)	0.25%			25° C.	Weekly or bi-weekly in daily proportions		Naumann	1925	Keeps for several months
Cow manure, fresh	½ Ash-can	Bean meal, fish meal (Struven's)	4 quarts 4 quarts	Outside	One in water in concrete pond 30 x 5 ft. in diameter— 1-2 ft. in depth	Good	Hayford (5)	1929	Resetting once a month Daphnia may be introduced 1 day after manuring
Cow manure, dried ("Bovung")— see table II	.01%	Marble-piece	Excess	21° C.	One time	Very good	Viehoever	1935	
Cow manure		Cooked oat meal					Hayford (5)	1929	Japanese method
Rat manure, fresh	.1%	Marble-piece	Excess	21° C.	One time	Good	Viehoever	1935	
Dog manure, fresh	.1%	Marble-piece	Excess	21° C.	One time	Good	Viehoever	1935	Amt. appar. excessive Reinoculation of Daphnia

TABLE I—Natural Manures (Continued)

Natural manures	Amount	Additions	Amount	T. ° C.	Applications	Results	References	Year	Remarks
Bird manure, fresh	0.25%				One time		Sjoested through Naumann	1922	Optimum concentration
Sheep manure, concentr.	0.1%					Very good	Naumann	1925	
Sheep manure, dried	6.0%				One :—to 1 gallon of distilled H_2O or to 1 gallon tap H_2O from 2-12 hours		Embody (12)	1928	
Sheep manure					Excessive application	Very good	Naumann (10)	1925	Self-purification produces medium for Green Algae and Flagellates
Pig manure					Excessive application	Very good	Naumann (10)	1925	Self-purification produces medium for Green Algae and Flagellates
Manure		Dead weeds, dead fish				Good	Hayford (5)	1929	For outdoor breeding
Human "Milorganite," dried	0.01%			21° C.	One	Poor to fair	Viehoever	1935	Reinoculation of Daphnia necessary
"Agrinite"	0.01%			21° C.	One	Poor to fair	Viehoever	1935	Reinoculation of Daphnia necessary
"Hynite"	0.01%			21° C.	One	Poor to fair	Viehoever	1935	Reinoculation of Daphnia necessary
Urine	0.4-1.6%			21° C.	One, after standing 4 days	Unsatisfactory	Viehoever	1935	

TABLE II

Sheep, Cow, Dog and Poultry Manures

		Water		Observation in days	Culture medium	2 animals added	Temp.	Remarks
Sheep manure Whizard brand (dried)	0.1 gm.	Tap	1000 cc.	After 1 day	No turbidity No color No odor		21° C.	
				After 4 days		2 large, 3 small, alive	21° C.	
				After 20 days	Clear, no scum	12 alive	21° C.	
				After 40 days		3 alive	21° C.	Unsatisfactory, too dilute
						10 animals added		
Sheep manure Whizard brand (dried)	1.0 gm.	Tap	4000 cc.	After 1 day			21° C.	
				After 3 days		7 animals, alive		Aspirator, applied
				After 5 days	Turbid	2 & 1 young, alive		Promising culture
				After 14 days	Slightly yellow	Many		
						2 animals added		
Sheep manure Whizard brand (dried)	1.0 gm.	Tap	4000 cc.	After 1 day			21° C.	
				After 13 days	Slight turbidity Slight odor Slight color	2 big, 24 small, alive		
				After 22 days		Many		
				After 26 days	Slight yellow color, no scum	11 big, 100 small, alive		
				After 40 days	Floc. Ppt.	Very many		Very good culture
						2 animals added		
Cow dung, (dried) "Bovung"	1.0 gm.	Tap	4000 cc.	After 1 day			21° C.	
				After 13 days		2 big, 12 small, alive		
				After 22 days		Many		
				After 26 days	Clear Slightly yellow	15 big, 35 small (Approx.)		
				After 40 days	No scum	Very many		Very good culture

Animals added after standing overnight

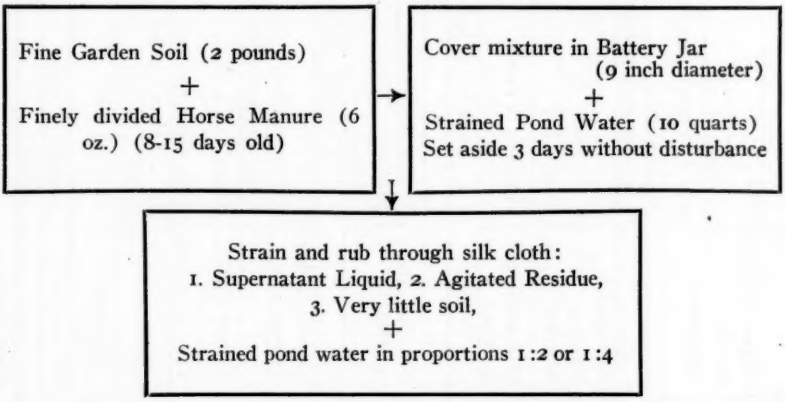
TABLE II—Sheep, Cow, Dog and Poultry Manures (Continued)

TABLE II—Sheep, Cow, Dog and Poultry Manures (Continued)

							10 animals added		
Cow dung, (dried) "Bovung"	1.0 gm.	Tap	4000 cc.	Animals added after standing overnight	After 3 days	Slight turbidity No odor Slight color	10 L.	21° C.	
					After 5 days		10 L. & 12 small (approx.)		Very promising culture
							2 animals added		
Cow dung, (dried) "Bovung"	0.1 gm.	Tap	4000 cc.	Animals added after standing overnight	After 1 day	No turbidity No odor No color		21° C.	
					After 22 days		Many alive		
					After 26 days	Floc. sediment No scum	About 20 or more		
							2 animals added		
Poultry, dried	0.1 gm.	Tap	1000 cc.		After 1 day			21° C.	
					After 4 days	No color, odor turbidity	2 big, 2 small		
					After 22 days		16 alive		
					After 40 days		All dead		Starved, unsatisfactory
							2 animals added		
Poultry, dried	1.0 gm.	Tap	4000 cc.		After 3 days	Slight turbidity Yellow color	2 animals alive	21° C.	
					After 13 days		4 big, 22 small alive		
					After 22 days	Yellow tinge Floc. Ppt.	Many		
					After 26 days	Very little scum	6 big, about 50 small		Good culture
							2 animals added		
Dog manure, fresh	1.0 gm.	Tap	1000 cc.		After 1 day		2 alive	21° C.	
					After 2 days	Very cloudy	1 alive, 1 dead	21° C.	Bacteria and protozoa
					After 3 days		2 dead	21° C.	(Excessive growth)
					After 5 days		5 added	21° C.	
					After 6 days	Clear, fairly	5 alive	21° C.	
					After 7 days		5 alive	21° C.	
					After 11 days	Slightly cloudy	5 & 10 small alive	21° C.	Good culture
					After 26 days	Almost clear	Very many	21° C.	Very good culture

representing a good culture medium; 3, even such a pond undergoes continually, and particularly in the different seasons, such changes from drying up or freezing to overflow that it's year-round usefulness for daphnia food becomes problematical. Banta therefore worked out a process of preparing a culture medium of material available practically anywhere and at any season.

BANTA'S CULTURE MEDIUM


Fine Garden Soil (2 pounds)
+
Finely divided Horse Manure (6 oz.) (8-15 days old)
Cover mixture in Battery Jar (9 inch diameter)
+
Strained Pond Water (10 quarts)
Set aside 3 days without disturbance
Strain and rub through silk cloth:
1. Supernatant Liquid, 2. Agitated Residue,
3. Very little soil,
+
Strained pond water in proportions 1:2 or 1:4

This soil-manure medium proved very useful in Banta's experiments and those of other workers as well as our own. The culture solution usually suffices the food requirements of an individual daphnia during its lifetime and consists mainly of bacteria as the food element. While a certain uniformity in the chemical and biological composition might be obtained, nevertheless such mass cultures are much too variable to be acceptable as a "Standard Food", so desirable in quantitative biological work.

MANURES—ANIMAL WASTE

An interesting survey of experiments, carried on by various workers, with natural manures under varying conditions is given in Table I, indicating that favorable results may be obtained with manures from various sources, provided, the amounts given at one time are small, and the number of applications of food is limited.

On Table II are the detailed observations enumerated of special comparative experiments with dried sheep manure, dried cow manure and dried poultry manure. The results appear quite conclusive that propagation at first is slow and somewhat uncertain, that the suitable concentration depends upon the conditions of the breeding method.

TABLE III

ANIMAL BY-PRODUCTS

	Amount	Additions	Amount	Applications	Results	References	Year	Remarks
Dead fish				One, in pond, breeding	Very good	Hayford (5)	1929	Replacing fish meal in bone meal cow-manure mixture
Fish meal	0.1%				Very good	Naumann (10)	1925	For pure cultures of Green-Algae & Flagellates; fodder organisms
Fish meal	0.1%				Very good	Naumann (10)	1925	Rich in N and P
(Struven's) Fish meal from menhaden fish	4 quarts	Bone meal Cow manure, green	4 quarts ½ ash can	One, in water in concrete ponds, 30 x 5 ft. diam., 1-2 ft. deep	Very good	Hayford (5)	1929	Resetting once a month Daphnia can be placed into ponds one day after manuring
Meat meal	0.1%				Very good	Naumann (10)	1925	For cultures of fodder-organisms for Cladocerae
Fish meal, herring	0.1%	Urine	1.0%		Very good	Naumann (11)	1925	Maximum amts. for Daphnia M. in presence of abundant Algae
Bone meal, green						Hayford (5)	1929	Also substituted by cooked oatmeal
Blood meal, dried	0.01%			One per week, never in large doses	Possibly best food and fertilizer	Naumann (9)	1925	"Apparently best for large numbers"
Blood (corpuscles) of birds or mammals					Good, depend, as in no other food, upon dosage	Wagler (13)	1925	Extraordinary tendency to putrefaction. Thorough aeration, renewal of culture water, addition of planaria or asellus—assist to overcome putrefaction, in removing waste products
Blood, fresh Blood meal, dried				"Little and often is the rule to follow" in mass breeding	Satisfactory	Anonym. author (14)	1933	"Forcing to Daphnia"

TABLE IV

BLOOD, MILK, MEAT SCRAPS AND BOUILLON

Food	%	Addition	Daphnia Magna	Added	1st day	2d day	3d day	4th day	5th day	6th day	7th day	9th day	11th day	18th day	Remarks
Meat scrap	0.01 (1L)	Marble-piece	2		2 L	2 L	2 L	2 L	2 L	2 L	2 L	2 L	2 L	2 L + 2 Y	Sl. cloudy at 3d day, clear on 5th
Blood	0.01 (1L)	Marble-piece	2		2 L	2 L	2 L	2 L	2 L	2 L	2 L	2 L	2 L + 1 Y	6 L + 15 V. Y.	Clear
Dried milk	0.01 (1L)	Marble-piece	2	5 added after 6 days	2 L	1 L 1 D	1 L 1 D	1 L 1 D	1 L	6 L	6 L	4 L	4 L	17 L + 5 Y. Y.	V. cloudy on 3d day, fairly clear on 5th day
Malted milk (Horlicks)	0.01 (1L)	Marble-piece	2			2 D	2 D								V. cloudy on 3d day, clear on 5th day
				5 added After 3 days			5 L				5 L + 2 Y	5 L + 3 Y	5 L + 8 Y	30 L + 12 V. Y.	Excellent culture
Meat bouillon "Bovril"	.066	Marble-piece	2		2 L		2 D								Cloudy 3d day, clearing later
				2 added after 5 days 2 added after 12 days 2 added after 23 days				10 L		2 D	2 D 2 D			Approx. 100 L	

L: Living. D: Dead. Y: Young. V Y: Very young.

TABLE IV—Blood, Milk, Meat Scraps and Bouillon (Continued)

Food	%	Addition	Daphnia Magna	Added	1st day	2d day	3d day	4th day	5th day	6th day	11th day	13th day	26th day	40th day	Remarks
Blood, dried	0.01 (1L)		2		1 L 1 D										After 1st day, no turbidity, color, odor
								1 L							After 4th day, pronounced odor
										1 L					
				1 added after 11 days							1 D				After 26th day, slight turbidity
												1 L			Yellowish color, reddish, flocculent sediment
													30 L	Very many	Very good culture

*Temp.: 21° C.

If factors as temperature, light, and volume as well as oxygen pressure, are kept fairly constant, then favorable results might well be expected within two to three weeks with any of the fairly uniform dried and shredded excrements, available in commerce as fertilizers. From work in progress now, it is obvious that artificial aspiration, lowering pollution and thus the number of casualties of daphnia, particularly in the beginning of propagation, greatly speeds the development.

Animal By-Products

On Table III is a survey of animal by-products used in the breeding of daphnia, again with satisfying success in the hands of the experimenter, who had by actual trials in indoor or outdoor breeding learned to use the optimum concentrations and the number of applications needed.

On Table IV are enumerated the special experiments and observations with animal by-products and foods or feeds as blood, milk, meat scrap and bouillon. The experiments with dried blood were particularly gratifying, after a number of initial failures, due to too high a concentration, too frequent application, too restricted an oxygen supply. The suitable dosage of bouillon seems somewhat difficult to obtain, although—in small quantities—it has given "excellent" result. (Aqu. Life.)(14) The experiments with peptone (.01%) are still inconclusive. As the solutions became cloudy, the animals died within three days.

Fertilizers

The data on Table V with various fertilizers in outdoor breeding of daphnia as forage for hatching fish, by the head of the Laboratory of Fish Culture, Cornell University, Dr. G. S. Embody and Dr. W. O. Sadler (15) of Mississippi College are self-explanatory. They are very helpful and suggestive as numerous quantitative data are provided. Hogan's experiments,(16) using cottonseed meal with success in pond breeding, are also of interest.

Plant Products

On Table VI are recorded the observations with plant products used as food or feed. While the data thus far available are too limited to permit definite conclusions, they indicate the probability that the concentrations chosen and the addition of small amounts of urea

TABLE V

FISH FOOD ORGANISMS PRODUCED IN CONCRETE PONDS 8 ft. x 12 ft. (Capacity: 2754 Lit.H_2O)
EACH SET REFERS TO THE PRODUCTION IN ONE POND FOR ONE PERIOD

Fertilizer	Stocking Quantity of Daphnia	Period in days	Production in Grams			
			Daphnia	Midges	Mosquitoes	Total
4 qts. sheep manure 1.5 pts. soy bean	?	15	695	150	0	845
4 qts. sheep manure 1.5 pts. soy bean	?	16	690	25	0	715
5 qts. sheep manure 1.5 pts. soy bean	?	20	407	309	442	1158
4 qts. sheep manure 1.5 pts. soy bean	?	21	462	77	0	539
4 qts. sheep manure 1 pt. soy bean	?	22	706	308	0	1014
1.75 pts. dry buttermilk	?	28	1318	75	152	1545
2 qts. cotton seed meal	50 cc.	21	800.0	56.0	0	856.0
4 qts. cotton seed meal	50 cc.	30	1280.0	60	0	1340.0
3 qts. cotton seed	?	30	1060	460	0	1520
1.25 qts. soy bean	100 cc.	16	1066	0	0	1066
1.25 qts. soy bean	25 cc.	17	1386	0	0	1386
2.25 qts. soy bean	100 cc.	17	686	358.4	0	1044
2.25 qts. soy bean	50 cc.	17	335	1092.16	0	1427
2.25 qts. soy bean	25 cc.	14	219	730.8	0	949.8
1.5 qts. soy bean	50 cc.	20	1759.5	369.4	0	2128.9
1.25 qts. soy bean	25 cc.	21	1460	246.0	0	1706.0
1.25 qts. soy bean	100 cc.	21	613.7	1104.0	0	1717.7
2 qts. soy bean	50 cc.	24	1825.0	174.5	416.5	2416.5

Data obtained by G. C. EMBODY and W. O. SADLER.

may yield a very simple and satisfactory culture medium. At least so far as soy bean flour, cotton seed meal and possibly also ground peanut is concerned. The beneficial effect of artificial aeration must be emphasized; as it was indeed striking.

FODDER ORGANISMS

Viosca (17) provides infusoria as a food for daphnia once or twice a day and feeds his infusoria with a bacterial culture about every second day (1 glassful per crock or tub).

TABLE VI—Soy Bean, Cotton Seed and Oat Meal

Food	%	Additions	%	%	No. Added	Subsequently	Observations 1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	11 days	Remarks
Soy Bean Flour	0.01	Ground Marble	0.01		4		3 L 1 D	2L 2D			2L	2L	2L	2L	2L 11Y	191 + on 31st day
" " "	0.01	" "	0.01	Urea 0.01	5		4 L 1 D	4L 1D					5D			Slimy Growth
" " "	0.02	" "	0.01		4		4 L V. active	4L			4L	4L	4L	4L		71 + on 31st day
" " "	0.02	" "	0.01		2	3	2 L V. active	1L 1D			5L	5L	5L	5L 1Y	5L, 3Y	Cloudy after 6 days No Aspirator 65 + on 30th day
" " "	0.03	" "	0 01	Urea 0.01	2	3	2 D	5D			5D					V. Cloudy after 3 day
" " "	0.02	Marble-Piece			3		3 L V. active	3L	3L	3L	3L	3L	3L	3L	1L	
" " "	0.01	" "			2		2 L V. active	2L	2L	2L	2L			5L, 5Y	5L 12Y	
" " "	0.0225	" "	0.02	Urea 0.0125	6	3 added 2nd day	1 L 5 D	2L 7D	2L	2L	2L	2L	2D			Heavy Sediment
" " "	0.0125	" "		" 0.0025	7		7 L						7D			No Art. Aeration
" " "	0.01	" "		Sheep Man. 0 01	2	4 added 6th day	2 L	2L	1L 1D	1L 1D			5L	5L 6Y	2L 6Y	Sediment greenish on 5th day—No Artif. Aeration 500 + on 32nd day
" " "	0.01	" "		Urea .00125	2	5 added 6th day	2 L				2D	5L	5L	5L	5L 2Y	510 + on 30th day
" " "	0.0125	" "		" .0025	10					4L 3Y	4L 3Y	4L 3Y	4L 3Y	4L 4Y	4L 11Y	Aspirator part time 450 + on 31st day
" " "	0.0125	" "		" .0025	10					6L 2Y	6L 5Y	6L 5Y	6L 12Y	6L 12Y	6L 26Y	Aspirator 3rd day 1000+on 30th day
" " "	0.006	" "	.006	" .003	1	1 added 5th day	3 L	3L	2L	2L	1L				0L	Unsatisfactory
" " "	0.006	" "	.006	" .003	2	2 " 5 "	2 L 1 D	3L 1D	2L		2D				0L	
" " "	0.012	" "	0.012	" .006	2	2 " 5 "	3 L	3L	3L		3D				0L	
" " "	0.012	" "	0.012	" .006			3 L	3L	2L	2L	2L				0L	
Cotton Seed Meal	0.01	" "			2				2L 1D	2L	2L	2L	2L	2L	2L	V. Goad on 17th day
" " "	0.02	" "				3 added 4th day			1L	4L		4L	4L	4L	4L	204 + on 34th day
" " "	0.02	" "			3		3 L	3L	3L	2L 1D		2L	2L	2L 5Y	2L 5Y	
" " "	0.01	" "		Sheep Manure 0 01	2	2 added 2nd day	2 D	2L 2D	2L 2D	2L 2D	2L	2L	2L	2L 3Y	2L 15Y	
" " "	0.01	" "		Urea 0.0025	10							2L 6Y			10L6Y	340 + on 30th day
Oat Meal	0.01	" "			3	3 added 7th day	2 L 1 D	2L	2L 1D	2L	2L	2L	5L	5L	5L 8Y	V. Goad on 17th day

L-Living Y-Young D-Dead.

INFUSORIA CULTURE

I

Water plants: Elodea, Covertail, Hornwort or Sagittaria, Vallisneria, etc., chopped (Hay or dry grass).

Small pieces (fresh: 2 oz.) + Pond water (1 gallon).

Small pieces (dried: ½ oz.) or Tap water (Cl. free) (1 gallon).

Plants decay (in few days); Bacteria scum forms (in 1-2 weeks) +

Living water plants or Pond water with some bottom mud } form infusoria in Scum

or

Inoculation with infusoria cultures } form infusoria in Scum

II

Water plants boiled with water (15 minutes). Tea-like infusion.

Set aside, uncovered, in dark place (few days).

Inoculate with paramecium or other protozoa culture.

Keep covered in diffused light: abundant infusoria.

BACTERIA CULTURE

Beef extract (5 teaspoonsful, boil with water) (1 gallon).

Cool, dissolve lye (1 teaspoonful).

Set aside, uncovered, in dark place.

Replace water, evaporating. Scum forms (1-4 weeks).

A. *Protozoa—Algæ.* The protozoa mentioned on Table VII have been described in literature as having served as food to various cladoceras, to which classification daphnia belongs. It is obvious from the observations of various workers that minute size and comparative freedom from protective membranes on the part of the protozoa are decisive factors in their serving as suitable food for daphnia.

The algæ, enumerated on Table VII, are, as indicated, not all acceptable for food. They must be minute, preferably one-celled and round, rather than filamentous without tough cellulosic or silicified membranes, in order not to interfere with the movement of daphnia in the cultures, in order to be readily digestible. Algæ often, seemingly spontaneously, occur in cultures exposed to the light. They grow much more abundantly in cultures which are water clear or only slightly colored than in darker colored culture solutions. Embody and Sadler preferred darker colored solutions of cotton seed meal, for outdoor breeding—as the growth of algæ was markedly reduced—in comparison with the excessive growth in clear mixtures made with soy bean. Naumann(11) found the presence of sugar, added to cultures of algæ, detrimental to daphnia. I too found that sugar in concentrations of .5 per cent. and 1 per cent. added to culture media,

TABLE VII—Fodder Organisms, Protozoa and Algae

Protozoa: Flagellates		Addition to culture water	Results	References	Year	Nutrient	Remarks
Chrysomonades	Living (naked)	Addition to culture water	Good, if right size	Naumann	1925		Readily digested by Cladocerae
Euglena	Living	Addition to culture water	Good, if right size	Wagler	1925		Readily digested by large Cladocerae
Euglena	Living	Addition to culture water	Apparently good	Embody	1928		Propagated with dried milk
Haematococcus Euglena Ankistrodesmus	Living	Addition to culture water		Embody	1929		Probable food for Daphnia
Heliozoa	Living	Addition to culture water		Woltereck (18)	1908		Food for Daphnia Longispina
Gymnodiniae	Living	Addition to culture water		Woltereck (18)	1908		Food for Daphnia Longispina
Paramoecium and Smaller Flagellates	Living	Present in culture water	Good	Viehoever	1935	Cotton seed Dog manure	Probable food for Daphnia
Smaller Flagellates	Living	Present in culture water	Good	Viehoever	1935	Sheep manure Blood Cow manure Horlicks Malted Milk	Probable food for Daphnia

TABLE VII—Fodder Organisms, Protozoa and Algae (Continued)

Algae		Addition to culture water	Results	Reference	Year	Nutrient	Remarks
Chlamydomonas	Living	Addition to culture water		Wagler (13)	1925		
Palmellaceae: Kirchneriella	Living	Addition to culture water		Wagler (13)	1925		
Chlorella	Living	Addition to culture water		Scharfenberg (19) (Knoerrich) (13)	(1901) 1925		(Recommended by some for Daphnia, not by others—Viehoever) Recommended for Cladocerae
Chromulina	Living	Addition to culture water		Woltereck (18)	1921		Recommended for Cladocerae
Cryptomonas	Living	Addition to culture water		Wagler (13)	1925		Recommended for Cladocerae
Mallomonas	Living	Addition to culture water		Wagler (13)	1925		Recommended for Cladocerae
Raphidium	Living	Addition to culture water	Unsatisfactory	Wagler (13)	1925		Thick shells or membranes prevent ready digestion
Scenedesmus	Living	Addition to culture water	Unsatisfactory	Wagler (13)	1925		Spiny extensions cause injury in intestines
Spirogyra: Fresh or dried	Triturated to fine paste	Addition to culture water	Unsatisfactory	Wagler (13)	1925		Incomplete destruction of cellulose membrane prevents ready digestion
Sphaerella-plurialis	Living	Addition to culture water		Woltereck	1921		
Protophytes:			Unsatisfactory	Naumann	1918		Pass intestine practically undamaged
With Silicic Acid membranes With tough cellulose membranes					1925		
Diatoms	Living	Addition to culture water	Unsatisfactory	Naumann			Indigestible
One-celled Green-Algae	Living	Addition to tap water	Fair, though inconclusive	Viehoever	1935	Algae grown separately in dilute sheep manure	Under further investigation.

TABLE VIII

ALGAE AND SUGAR

Fodder-Organisms	Saccharose	After 24 hours	After 6 days	After 14 days	Remarks
Algae	5 %	Daphnia, dead			Addition of glucose (.5% and 1%) to culture media caused growth of fermentative organisms, formation of CO_2 and death of Daphnia in my own experiments. —Viehoever
Algae	2.5%	Daphnia, normally active	Dead		
Algae	0.5%	Daphnia, normally active	Infected with various sewage-molds, sewage-bacteria and vorticellids	Dead	

Observations made by Naumann.(11)

caused increased growth of fermentative organisms with formation of carbon dioxide and death to daphnia. (See Table VIII.)

B. *Bacteria—Yeast.* The use of bacteria for food has been suspected or emphasized by the various workers who have observed the conditions under which daphnia live and breed, in nature as well as in the laboratory. Treillard (20) as one of the first, claims that he cultivated daphnia in pure cultures of bacteria. Stuart, McPherson and Cooper of Brown University (21) have carried on very valuable work with the crustacean-moina macrocarpa, closely related to daphnia. Their comprehensive work is indeed suggestive. (See Table IX.)

Believing that in many of the manural culture media urea bacteria would be present and thus serve as bacterial food to the daphnia—a mineral media containing urea was prepared, consisting of .02% urea, .05% Liebig Extract, added to an aqueous solution of $K_2H\ PO_4$ .1%, $CaCl_2$, .01%, $MgSo_4$, .03%, NaCl .01%, $FeCl_3$ .001%. Urea bacteria grew readily in this solution.(22) While the sterile solution in various concentrations was unsatisfactory, two drops of this selective urea medium with urea bacteria, added daily to 30 cc. cultures of tap water, exposed to direct and diffuse light, gave fair to good growth of daphnia. Some of these cultures showed the development of one-celled green algæ in addition to urea bacteria and particularly good growth. Also

TABLE IX**

FODDER ORGANISMS—BACTERIA FOR MOINA

Species	Gen. Reared	No. Separate Exper.	Total No. Anim. Tested	Died	No. Anim. Rel'g Young	Av. Young per Clutch	% Anim. Prod'g Young	% Living Animals Uncounted
Normal medium	1	5	68	17	51	6.2	75	....
	2	3	14	3	11	7.6	78.5	....
	3	2	3	1	2	10.5	66.6	....
Bacillus coli mutabole	1	4	46	1	44	7	95.6	97.8
	2	4	25	11	13	8	52	92.9
	3	2	3	2	1	10	33.3	100
	4	1	1	0	1	14	100	100
	5	1	1	0	0	0	0	0
Aerobacter aerogenes	1	2	11	1	10	3.3	90.9	100
	2	2	8	0	7	10.7	87.5	87.5
	3	2	5	2	1	12	20	33.3
	4	1	1	0	1	8	100	100
	5	1	1	0	1	17	100	100
	6	1	1	0	1	15	100	100
Staphylococcus albus	1	1	6	1	4	1	66.6	80
	2	1	3	0	3	8	100	100
	3	1	2	0	2	10	100	100
Alkaligenes fecalis	1	1	6	1	4	2	66.6	80
	2	1	4	1	2	9	50	66.6
	3	1	2	2	....	...	....	No test
B. subtilis	1	1	6	4	0	0	0	0
Sarcina luteus	1	1	6	3	3	1	50	100
	2	1	3	0	3	8	100	100
	3	1	3	0	3	7	100	100
Spirillum rubrum	1	1	5	1	4	6	80	No test purity
Chromobact. violaceum	1	1	6	6	....	...	....	6 pure
Unknown 1*	1	1	6	6	....	...	....	2 pure
Unknown 2*	1	1	6	6	....	...	....	3 pure
Unknown 3*	1	1	6	3	3	2	50	100
	2	1	2	1	1	10	50	100
	3	1	1	1	....	...	....	1 pure
Mixture	1	2	16	13	3	5	18.7	No test

*Isolated from normal medium.

**Data obtained by Stuart, McPherson and Cooper.

an old manural degenerated solution of sheep manure could be invigorated for daphnia growth with the special urea bacteria culture medium.

Further work along this line is in progress.

The value of yeast in feeding daphnia has been previously mentioned by me in an article on yeast,(23) wherein I pointed out that it facilitated their breeding. The experiments, then conducted in an exploratory survey, are now being continued, using suspensions of .01 per cent. and .005 per cent. of fresh yeast cake in water. The daphnia are placed in these suspensions, containing also an excess of calcium carbonate in the form of marble, and their speed and extent of propagation is noted. These interesting experiments are also under way.

8. Exclusion of Enemies

Daphnia is beset with a host of enemies. As we stated previously,(24) we have observed the fish mold "saprolegnia", related to the well-known fly mold, infest and subdue daphnia cultures, especially when the medium became acid; we have seen rotifers infest and overcome the daphnia; we have observed related crustaceæ, like cyclops, crowd out the daphnia and we have seen the well-known hydra first paralyze and then—at leisure—destroy daphnia irrespective of their size and vigor before the contact; we have witnessed worms as the aquatic earthworm or simple forms as waterpolyps or even the one-celled protozoa stentor devour young daphnia. We all know how eagerly various kinds of fishes hunt them for food.

These enemies, fortunately, can be rather readily removed, either by actual separation from the culture medium, in which they may be observed, or by avoiding conditions as acid media and pollution, favoring certain enemies. Of various means to kill hydra—the method, suggested by Dr. Roth, has been specially recommended, namely the immersion of the entire strained catch or culture in 1 per cent. salt solution for one or two minutes. Of course, the filtering of pond water for use in cultures is always advisable as a preventative measure.

9. The Selection of a Pure Line Stock of Animals

Having satisfied all the requirements for the successful propagation, the selection of a pure line, so desirable in quantitative biological work, is the final step. This is greatly facilitated by the ready

TABLE X

YEAST AS FODDER ORGANISM

Yeast (fresh) X R (Fleishmann's) %		Second addition of yeast after 4 days %	Initial addition	%	No. of Daphnia added	Subsequently added after 4 days	1st day	2d day	3d day	4th day	5th day	Remarks
Yeast	.01	.01	Marble	Excess	2	2	2 L	2 D	2 D		2 L	Supernatent liquid clear after 2 days, fairly heavy sediment
Yeast	.02	.01	Marble	Excess	2	3	2 L	1 L 1 D	2 D		3 L	
Yeast	.01	.01	Marble	Excess	2	2	2 L	1 L 1 D	2 D		2 L	
Yeast	.01	.01	Marble	Excess	2	2	2 L	2 L	2 L	2 D	2 L	
Yeast	.01	.01	Marble	Excess	2	2	2 L		2 D		2 L	
Yeast	.005	.005	Marble	Excess	2	2	2 L	2 L	1 L 1 D	2 D	2 L	
Yeast	.005	.005	Marble	Excess	2	2	2 L	2 L	2 L	2 L	3 L	
Yeast	.005	.005	Marble	Excess	2	2	2 L	1 L 1 D	2 D		2 L	
Yeast	.005	.005	Marble	Excess	2	1	2 L	1 L 1 D	1 L 1 D	2 D	2 L	
Yeast	.005	.005	Marble	Excess	2	1	2 L	2 L	2 L	1 L	2 L	
Yeast	.005	.005	Marble	Excess	2	1	2 L	2 L	2 L	1 L	3 L	

and frequent formation of eggs ("summer eggs") developing, within a few days,—by parthenogenesis—into adults, who in turn may be selected as the pure line breeders.

Inasmuch as many eggs—up to fifty—may be released at one time—from the ovary of the females into the brood sac for development until birth, to be followed shortly by other sets, the experimental animals thus obtained greatly excel the usually available experimental animals,—in uniformity. In addition to the fact that they all come from the same mother,—if need be,—they have the same age and thus lend themselves remarkably well for biological studies. There is but one difference—and this does not interfere with our results, if we base them on a large number of animals (sisters),—namely the individual variation in vitality, common to all forms of life, but less marked in descendants of the same stock.

Conclusions

1. The propagation of Daphnia Magna for laboratory experiments is assured by extremely simple methods, merely adding certain animal or plant products, commonly available, without special preparation, to water (tap, or distilled), suitable for drinking purposes (practically free of chlorine and heavy metals), alkalinized with an excess of calcium carbonate (coarsely crushed marble stone), or precipitated chalk, preferably aerated and kept at room temperature (21° C.) in the diffuse light of northern exposure.

2. The cultivation of consecutive generations of suitable experimental animals (females developing parthenogenetically summer eggs),—in agreement with the experience of other workers, may be assured with any of the common natural manures of animal excretions, provided, 1, the amounts given at one time are not excessive, as otherwise causing putrefaction; 2, the applications are frequent enough to prevent starvation; 3, the accumulation of toxic substances is prevented through fairly frequent (monthly or bi-monthly) renewal of cultures and artificial aeration, if necessary; 4, a sufficient number of vigorous animals is used to start the daphnia culture.

3. Dried, shredded sheep manure (Whizard Brand) in proportion of 0.025 per cent. (maximum) has proven very satisfactory; the same concentration of dried shredded cow-manure ("Bovung") gave equally satisfactory results.

4. Of various animal by-products, dried- and malted milk (Horlick's), and dried pig blood in amounts of 0.01 per cent. (maximum) gave best results, particularly after standing and self-purification of the culture media.

5. Of plant products, cotton seed meal, found satisfactory in large scale, out-of-door breeding by Hogan (1933) and Embody (1935), is very good for laboratory breeding in amounts of 0.01-0.02 per cent. (maximum). Excellent results prove experiments with soy bean flour in amounts of 0.01-0.02 per cent. (maximum), in agreement with Embody's results feeding soy bean meal in large scale pond experiments. The addition of urea in amounts of approximately .003 per cent. (maximum) appears advantageous.

6. Of micro-organisms, raw and pure cultures; infusoria, algæ, bacteria and yeast, those of bacteria appear most satisfactory for laboratory breeding of daphnia; the value of certain common bacteria used as exclusive food, as claimed in previous work of Treillard (1925) and Stuart (1931) for Moina, is indicated in my preliminary work with urea bacteria.

7. The host of enemies, which may destroy daphnia, can be rather readily kept out of cultures or, if found present, usually quickly removed.

8. The easily accomplished selection of a pure line stock, and the fact that from it many descendants may be obtained (by parthenogenesis) at one time, as well as in subsequent births—over 1000 within 1 month, starting with 6 animals—assures unexcelled uniformity in age, size and vitality, so desirable in quantitative biological work.

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Blood Transfusion

> "Imbecile, now listen to what I have seen. When a soldier bleeds from a wound in battle these leeches say 'Fever! Blood him!' and so they burn the wick at tother end too. They bleed the bled. Now at fever's heels comes desperate weakness; but these prickers and burners, having no forethought, have meantime robbed him of the very blood his hurt had spared him to battle that weakness withal; and so he dies exhausted."

Thus in the words of Denys, in *The Cloister and the Hearth*, we have open rebellion against the venerated practice of blood-letting, which, in the fifteenth century, was a far more universal procedure by physicians than the conserving or replenishing of it.

About 200 years later, in 1667 to be exact, another Frenchman, oddly enough by the same name, became sufficiently skeptical of the therapeutic value of removing blood to try the then novel experiment of furnishing to the depleted vessels of the sufferer a fresh supply of it.

Jean Baptiste Denys, physician to Louis XIV, had occasion to visit a child dying of repeated bleedings performed for the relief of some obscure ailment. Into the veins of the youth he caused to be injected lamb's blood, with immediate and beneficial results and the ultimate recovery of his patient.

SCIENTIFIC AND TECHNICAL ABSTRACTS

Compiled by Arthur Osol, Ph. D.

Dispensing Notes and Difficulties. Chemist and Druggist, 122, 169 (1935). The following prescription was submitted with the statement that when it was first filled no deposit was observed to the final dose but that the second bottle was found to contain a white precipitate after standing for two days. The inquirer states that he compounded the ingredients identically in both cases and used the same quality of drugs.

Potassium Citrate	4 drachms
Calcium Lactate	2 drachms
Liq. Adrenalin	30 minims
Syrup of Lemon	1 ounce
Chloroform Water, to make	8 ounces

In comment, it is pointed out that citric acid and citrates are reagents for the precipitation of calcium, and soluble calcium salts are reagents to distinguish between tartaric and citric acids, the former being precipitated in the cold, the latter by boiling. Potassium citrate and calcium lactate in concentrated solution react quickly, and in dilute solutions upon standing. If the lactate is dissolved in hot water and the citrate added, a heavy precipitate forms immediately. To dispense the mixture, cold solution is recommended, as follows: Triturate the calcium lactate with 1½ drachms of compound powder of tragacanth and 2 ounces of chlorform water. To this add the other ingredients and dilute to 8 ounces with chloroform water.

Attention is called to the fact that old calcium lactate varies considerably in solubility and also that the addition of mucilage of tragacanth prevents precipitation from solutions which are not too concentrated.

Calcium Thiosulphate. P. Petrovici. *Journ. Pharm. et Chim. 19,* 392, (1934). Crystalline calcium thiosulphate, containing six molecules of water of crystallization, may be prepared in the following manner. A solution of 100 grams of calcium chloride in 32 grams of water is warmed to approximately 38 degrees C. and 140 grams of sodium thiosulphate added in small portions at a time. Calcium

thiosulphate is formed by double decomposition, the reaction being complete in half an hour. The precipitate is collected on a Büchner filter, previously warmed to 50 degrees C., and allowed to dry in the air. Traces of sodium chloride may be removed by recrystallization. Calcium thiosulphate hexahydrate is fairly stable in air, decomposing only very slowly with the formation of calcium sulphite and sulphur. Upon heating to 30 degrees C. the hexahydrate is converted to monohydrate, the latter also being obtainable by drying the former over sulphuric acid. At temperatures above 30 degrees C. decomposition occurs; water, sulphur and sulphite being formed.

Detection of Glycerin in Mixtures. K. Täufel and H. Thaler. *Pharm. Zeitung (Berlin) 79,* 341 (1934). Glycerin may be detected by converting it to epihydrin aldehyde which produces a red to violet color with phloroglucin. The solution under test is evaporated on a water-bath and the residue treated with 2 to 3 grams of 40 per cent. milk of lime which prevents interference of carbohydrates with the test. The mixture is again evaporated and the residue extracted with three portions of 4 cc. each of absolute alcohol. This solution is evaporated and the residue dissolved in 3 cc. of a mixture of equal volumes of ether and absolute alcohol. After filtration and washing with a 3 cc. portion of ether-alcohol, the solution is transferred to a small flask, evaporated, and the residue treated with 15 drops of phosphoric acid. After adding pumice the mixture is distilled to effect separation from allyl alcohol, eugenol and geraniol which also interfere with this test. The distillate, which amounts to but a few drops and which contains acrolein, is treated with a drop of 3 per cent. hydrogen peroxide and 1 cc. of concentrated sulphuric acid. After cooling, the mixture is shaken vigorously for one minute, the excess of peroxide decomposed by means of a drop of potassium iodide solution and decolorized with sodium thiosulphate. The resulting solution is mixed with 0.5 cc. of 0.15 per cent. solution of phloroglucin in ether which produces a red to violet color if epihydrin aldehyde is present. In the presence of small quantities of the aldehyde the color develops slowly.

The test is sensitive to 100 γ or less of glycerin. If sucrose or raffinose is present, they must be first inverted by warming the residue resulting from the first evaporation with 5 cc. of 4 per cent. sulphuric acid.

New Remedies. Abstracted in part from *Quart. Journ. Pharm. Pharmacol.* 7, 760 (1934). *Bee Venom* is a preparation of the venom of bee-stings for the treatment of rheumatism. The major part of the protein is fixed and discarded during the process of manufacture and the venom is suspended in normal saline containing 0.5 per cent. of phenol. Bee venom is supplied as a course of 12 ampoules containing progressively larger doses. The first ampoule contains the equivalent of half a normal bee-sting, this dose being known as five units. The injection should be given well under the skin, when there will be but slight local reaction.

Forapin is a bee-venom salve supplied for the treatment of neuritis, arthritis, rheumatism and other conditions requiring a counter-irritant. It is claimed that the application of bee-venom in the form of an ointment is equal in effect to the older method of injection, and its application is simple and almost painless. It is possible to introduce large amounts of venom in chronic cases without causing any considerable pain. The bee-venom is procured from living bees. Forapin is supplied in tubes in two concentrations, I normal, and II strong.

Jelopar Nasal Jelly contains sodium ricinoleate (1 in 400) and menthol in a petroleum jelly base. It is claimed that the slightly alkaline sodium ricinoleate softens the nasal mucus without causing irritation, and by lowering the surface tension enables the oily medium to penetrate the recesses of the mucous membrane. Jelopar nasal jelly is recommended for the prevention and abortive treatment of colds and influenza. It is supplied in 25 cc. and 50 cc. tubes.

Sedonan is a 5 per cent. solution of phenazone in anhydrous glycerin, for use as ear-drops in inflammatory conditions. It is particularly recommended in otitis media before perforation has occurred. After perforation it should not be used if the discharge is purulent. The relief of pain and reduction of inflammation are due to osmosis. The drops should be used cold, and hot or wet poultices should not be applied.

Alkaloids of Opium. J. H. Stadelmann. *Pharm. Tijdschr. v. Ned.-Ind.*, 11, 33 (1934). Through *Quart. Journ. Pharm. Pharmacol.*, 7, 674 (1934). An account is given of the chemistry and structure of the alkaloids of opium. The table below summarizes the twenty-five alkaloids which have been reported in opium, where they occur combined with meconic acid, lactic acid and sulphuric acid.

Alkaloid	Mean percentage in opium	Empirical Formula	M. Pt.	$[\alpha]_D$	
Morphine	9	$C_{17}H_{19}O_3N$	253° C.	—133°	
Codeine	0.3	$C_{18}H_{21}O_3N$	155° C.	—135°	
Thebaine	0.4	$C_{19}H_{21}O_3N$	193° C.	—218°	
Pseudomorphine	0.02	$C_{34}H_{36}O_6N_2$	Decomp.		
Papaverine	0.8	$C_{20}H_{21}O_4N$	147° C.	0	
Laudanosine	0.008	$C_{21}H_{27}O_4N$	115° C.	+108.4° (in HCl)	
Laudanine	0.01	$C_{20}H_{25}O_4N$	166° C.	0	
Laudanidine		$C_{20}H_{25}O_4N$	177° C.	—87.8°	(Laevolaudanine)
Narcotine	5	$C_{22}H_{23}O_7N$	176° C.		
Gnoscopine		$C_{22}H_{23}O_7N$		0	(Racemic narcotine)
Narceine	0.2	$C_{23}H_{27}O_8N \cdot 3H_2O$	170° C.		
Cryptopine	0.08	$C_{21}H_{23}O_5N$	218° C.	0	
Protopine	0.003	$C_{20}H_{19}O_5N$	200° C.	0	
Codamine	0.002	$C_{20}H_{25}O_4N$			
Lanthopine	0.006	$C_{23}H_{25}O_4N$	141° C.		
Meconidine		$C_{21}H_{23}O_4N$	58° C.		
Neopine		$C_{18}H_{21}O_3N$		+18.85° (in HCl)	
Pseudopapaverine		$C_{21}H_{21}O_4N$			Now known to be identical with papaverine
Rhoeadine		$C_{21}H_{21}O_6N$	232° C.		
Porphyroxine		$C_{19}H_{23}O_4N$	125° C.	—134.5° (in HCl)	
Tritopine	0.002	$(C_{21}H_{27}O_3N)_2O$			Now known to be identical with laudanidine
Xanthaline		$C_{37}H_{36}O_9N_2$			Oxidation product of papaverine
Oxynarcotine		$C_{22}H_{23}O_8N$			
Hydrocotarnine		$C_{12}H_{15}O_2N$			
Papaveramine		$C_{21}H_{21}O_5N$			

An account of the color reactions of the different alkaloids is also given.

SOLID EXTRACTS

By Ivor Griffith, Ph. M., Sc. D.

"Water, water, all around—nor any drop to drink" may ring true to the derelict tied to a raft, but the modern ocean liner, as it rides the ocean blue, distills its own pellucid water.

The purest natural water is not sufficiently free of mineral salts for use in the boilers of modern ocean liners. On some ships, 500 tons of water are required every twenty-four hours, all of which must be distilled. If any undistilled water, even a cupful, accidentally gets into the supply lines, an automatic alarm immediately notifies the engineers.

Close your eyes and ask someone to place upon the palm of your right hand two large size pills. With the fingers of your left hand, eyes still closed, touch the pills. If they feel like a pair of dice, you have *astereognosis*. A number of persons are afflicted with this curious condition, which is believed to be brought on by a cerebral dysfunction. It is the inability to recognize the size, shape or nature of a solid object by the sense of touch. Such a person, when blindfolded, cannot name an article that is handed to him.

But if you repeat the performance, crossing your two fingers of the left hand, then roll just *one* pill held in the right palm, under the crossed finger tips, and if you get the feeling of two pills—you are *normal*.

There is a Bohemian tradition to the effect that young maidens occupied in picking flax in the field become most alluringly beautiful and noted particularly for their fairness of skin. In keeping with all traditions that are believed in by a civilization from generation to generation, an element of fact is always discernible if it be intelligently sought. The "foolish" tradition of Pliny's time concerning the powerful medicinal value of the toad skin was made erudite by the findings of Abel at Johns Hopkins University. The "silly" feeding of cat's liver by Galen to dispel the sickly pallor of a "bewitched" person was turned into fact by the findings of Whipple at Harvard who

has demonstrated the value of that tissue in the treatment of anemias. So perhaps with the Bohemian tradition concerning flax.

More than likely, the Bohemian damsels, through a constant nibbling at the dainty flaxseed—with its content of colon-cleansing mucilage—kept their systems clear of poisons, and thereby found a fairness and a healthfulness of skin.

"Painting the lily"—is now an accepted performance—even in the field of everyday foods. French peas, were once so vitriol tinctured to their green, that every pea that rolled down papa's knife left a trail of copper in its wake.

But the British have a better idea.

The coloring of fruit and vegetables is receiving the attention of some of the master minds in the British dyestuffs industry, who have been concentrating on overcoming some of the difficulties encountered by the canning industry. The primary object of giving the foods a more attractive appearance is already being attained, but the dyestuffs tend to remain in the liquor in the cans, and may show a different color from the goods themselves. The consumer does not like this and has found fault, particularly with peas.

The chemists of Imperial Chemical Industries, Ltd., claim to have overcome these objections by the use of dyestuffs in the presence of an organic acid. In the canning of peas, for example, they propose the use of Lissamine Green V together with tartaric acid, a dry mixture of the dye and acid being used. This is made by mixing 95 parts of the dyestuff with 5 parts of tartaric acid, and this preparation is used in making the canning liquor.

Thyro-tips, a thyroid-containing cigarette, is the latest contribution of the advertising art to the business of weight reduction. The thyroid extract is painted over the cork tip and the would-be-reduced thus lip-absorbs the medication, which is not spoiled by the combustion of the cigarette. Needless to state this is a ridiculous, uncontrollable way of exhibiting thyroid extract, and although the package reads something like this: "To be used under a doctor's supervision," it is obvious that they can be bought like any other cigarette, by carton or carload. Watching the "feed limit" is a much safer way to girth control.

The gravimetric evaluation of the brain's capacity to think is now alleged to be readily demonstrable. The afflux of blood to the

brain during mental activity has been demonstrated by having a freshman student lie on a delicately balanced table that would tip downward at either end if the weight on it was increased. When the freshman's mental activity was at a low level the table did not move. But when he set his mind at work on an arithmetic problem the head-end sank at once under the increased weight.

—And so—"a penny-weight for your thoughts."

Rubefacients and counter-irritants have been used since the oldest days—in the treatment of all manner of ailments. The mustard plaster, onion poultice, ant-sting and bee-sting, and blisters galore, all are yet used as home remedies. But here is the oddest of panaceas. Moxibustion still is one of the popular remedies of Japan, being considered efficacious in all kinds of illnesses from rheumatism and eczema to headache and fainting spells. The doctor just places a pile of moxa leaves on the affected part of the body and sets it on fire, that is, the pile of leaves. The spot gets burned—and well!

The Greeks had a simple sort of a chemistry—four elements, air, earth, fire and water, and the quint-essence—Life. From four to Moseley's ninety-two was quite a step, and ninety-two elements seems a big enough variety to build any universe. But listen—The census of isotopes kept by Prof. Aston, the Cambridge, England, physicist, shows that 247 stable element varieties are now known from 79 of the 92 elements.

Isotopes in chemistry correspond roughly to non-identical twins in animals, since they are the same stuff but the atom of one isotope has a different mass or weight than another isotope of the same element. Oh, my!!

"Like Cures Like"

Solomon is reputed to have been the author of the saying that "there is nothing new under the sun," and it is interesting to know that one of the most recent methods of treating "ivy poisoning" has been a tradition of the populace for hundreds of years. That is, the idea of developing a resistance to the poison. It is asserted that the American Indian had evolved the idea of chewing the leaf to lessen susceptibility long before the advent of the "paleface" on this continent.

BOOK REVIEWS

THE ROMANCE OF EXPLORATION—Many a gallant and memorable figure stalks across the pages of "The Romance of Exploration and Emergency First-Aid", a booklet just issued by Burroughs, Wellcome & Co. Indeed, it is far more than just a booklet. It is a useful addition to the library of any man who is not already a specialist in this fascinating subject.

For here you will find the high spots in the careers of most of the great modern explorers. Ostensibly it covers only the period from Stanley to Byrd. Actually the part devoted to Africa begins with James Bruce in the XVIII Century, the pages dealing with Arctic travel start with Eric the Red and those telling of high adventure in the Antarctic include sketches of Captain Cook and the Russian Admiral Bellingshausen.

In effect, this is a compendious handbook of most of the important achievements in discovery in the last two centuries. It surveys rapidly the amazing histories of Stanley and Livingstone in Africa and also tragedy of Dr. Mungo Park. Burton and Speke, Grant and Sir Samuel Baker, Emin Pasha and the Baron Dhanis are other heroes of African adventure to be found in this little volume.

Among other dramatic narratives to be found in "The Romance of Exploration" are those of Sir John Franklin and the intrepid fellows who went to the Arctic to find him. Likewise the tale of what happened to General Adolphus Washington Greely. Peary, of course, Nansen and Andree, Walter Wellman and Vilhjalmur Stefansson.

Naturally, the accomplishments of Admiral Byrd are duly related, as well as those of Scott and Shackleton, Amundsen and Sir Hubert Wilkins.

Here, too, we encounter the Roosevelts, father and sons, Roy Chapman Andrews and Sven Hedin, the Duke of the Abruzzi and Dr. Alexander Hamilton Rice. One portion of the booklet deals with the conquest of Mount Everest and another with the pioneers of aerial navigation, from Bleriot to Lindbergh.

It is only natural that the booklet should drive home one salient factor in all voyages of discovery; the importance of compact and reliable medical equipment. In this it only bears out what one can learn from the memoirs of the great explorers who, better than any one else, know that having an efficient, compact medical equipment may mean the difference between achievement and disaster.

The book is profusely illustrated with reproductions of the medical and first aid equipments carried to the four quarters of the globe by those intrepid souls who matched their skill, strength and dauntless courage against the malignant forces of nature. In reading the book one's imagination is stirred by the realization that these medical outfits actually have been carried all over the world and have the honor and distinction of being the only medical kits to have reached both the North Pole and the South Pole on several separate occasions and to have gone around the word by air.

The collection of relic medical equipments which formed a highly interesting part of the Burroughs, Wellcome & Co. exhibit in the Hall of Science at the Chicago World's Fair now may be seen at the firm's Exhibition Galleries in New York.

THE POPULAR PRACTICE OF FRAUD, by T. Swann Harding, Longmans Green and Company, London, New York, Toronto, 1935. A book in a red cloth cover, with a yellow jacket, screaming "Fraud" from thirteen different vantage points on the front face of the jacket. Formally described as follows: 376 pages, 21.5 x 14cm.; cloth, $2.50.

Next to himself, T. Swann Harding is one of the reviewer's favorite authors.

He is interesting, witty and thorough. When he "takes up his pen" or "attacks his typewriting machine", he has a worthwhile tale to tell.

There must be some unwritten law of publishers that, when they first publish an author's work, they ignore everything that he has previously published, or perhaps they may think they have just discovered a new and budding writer.

But, perhaps this neglect in furnishing necessary information as to past achievements is due to the author's innate honesty, so, for the information of Longmans Green and Company, and as a reminder to the author himself I should like to inform those who are about to purchase and read this book, which reading I consider necessary for the proper understanding of our modern civilization, that the author has previously written three other interesting books called respectively: "Fads, Frauds and Physicians"; "The Degradation of Science", and "The Joy of Ignorance."

In all of these, as well as in the present volume, there is a central motif—the information of the public concerning many "quasi" and "pseudo" scientific claims and beliefs and the exposure of fads and fallacies.

In the present volume the author deals with the many direct and indirect ways in which the "John Does" and "Richard Roes" together with their wives, children and grandchildren are cheated, deceived, swindled, imposed upon and tricked, as well as being subjected to dishonesty, duplicity and imposition in many cunning ways which are peculiar to our time and our civilization.

Some time ago I was talking to a man who is prominently connected with a well known advertising firm. Happening to have a copy of the "Joy of Ignorance" on my desk I asked him if he had read it. He replied "No!" I asked "Why not?" His reply was to the effect that he dared not read the present-day literature which criticises advertising because he would not have faith in his work and could not continue to write or approve "convincing", "sales compelling" copy.

Now here is another book by T. Swann Harding, brimful of information for "John Q. Public" and his family.

Listen to some (but not all) of the chapter headings:

I	The Consumer Goes to Market.
IV	Fraud and Scientific Progress.
V	Hell Fire Advertising.
VIII	Nostrums Queer and Quaint Devices.
X	Sucker Trapping by Mail.
XII	So They Gave Their Testimony.
XIV	Can We Afford Our Frauds?
XVII	The Federal Government and the Consumer.
XX	The Future of Fraud.

There are notes, which will bore some persons, but which need not be read by those who are not interested in source material.

Unfortunately there is no index, but this omission can be appreciated by those who have prepared indexes, for there are so many items of importance that if an index of value and completeness had been prepared it might have delayed the publication of the book for some weeks, which would have been a loss to the public.

The thinking individual who reads this book will have no doubt of the necessity for new and adequate legislation for foods, drugs, cosmetics, etc., which shall make it illegal to broadcast or to publish in any way, shape or form any statements regarding a product or an article which would be illegal if made upon the label or if printed in the literature accompanying the package.

CHARLES H. LAWALL.